

AL/OE-TR-1995-0009
VOLUME I of III



ARMSTRONG
LABORATORY

**GENETIC TOXICITY EVALUATION OF
IODOTRIFLUOROMETHANE (CF₃I)**

**VOLUME I: RESULTS OF *SALMONELLA*
TYPHIMURIUM HISTIDINE REVERSION ASSAY
(AMES ASSAY)**

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FINAL REPORT FOR THE PERIOD MARCH THROUGH DECEMBER 1994

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TECHNICAL REVIEW AND APPROVAL


AL/OE-TR-1995-0009
VOLUME I

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER


TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

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PREFACE

The U.S. Air Force is investigating chemical replacements for the fire suppressant/ extinguishant Halon 1301. Iodotrifluoromethane (CF₃I) is closely-related structurally to Halon 1301 (CF₃Br) and may serve as a "drop in" extinguishant replacement. Results from laboratory animal *in vivo* studies indicated that CF₃I has a low order of acute toxicity. A comprehensive literature search indicated that no information was available on the mutagenic potential of CF₃I. ManTech Environmental initiated a battery of three short-term assays that were utilized to assess the mutagenic and clastogenic potential of CF₃I. Protocols for these assays were in conformance with the Environmental Protection Agency's (Toxic Substance Control Act) Health Effects Testing Guidelines.

This document, Volume I of III, serves as a final report detailing the results of the salmonella typhimurium histidine reversion assay (Ames assay) in the genetic toxicity evaluation of CF₃I. Volumes II and III will describe, respectively, the results of the mouse bone marrow erythrocyte micronucleus test and the results of the forward mutation assay using L5178Y mouse lymphoma cells.

The research described herein began in March 1994 and was completed in December 1994 by Genesys Research, Inc., Research Triangle Park, NC, under a subcontract to ManTech Environmental Technology, Inc., Toxic Hazards Research Unit (THRU), and was coordinated by Darol E. Dodd, Ph.D., THRU Laboratory Director. This work was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, and was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F30). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

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SUMMARY

Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated tested iodotrifluoromethane (CF₃I) using Billups-Rothenberg exposure chambers for the exposure chamber modification of the *Salmonella typhimurium* histidine (*his*) reversion mutagenesis system (the Ames test), a microbial assay that measures *his*⁻ → *his*⁺ reversion induced by chemicals that cause base changes or frameshift mutations in the genome of this organism. Testing was conducted using five *Salmonella* strains, with and without metabolic activation.


CF₃I was tested in a preliminary test, which was repeated because of excessive toxicity, and in a mutagenesis test. When tested over a concentration range of 1,060 to 85,908 ppm, CF₃I was found to be highly mutagenic, inducing frameshift and, particularly, base-pair mutations in *Salmonella typhimurium*, without and with activation. Therefore CF₃I was positive in the *Salmonella typhimurium* histidine (*his*) reversion mutagenesis assay.

**GENESYS RESEARCH INCORPORATED'S
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

**MUTAGENESIS TESTING OF IODOTRIFLUOROMETHANE (CF₃I)
USING THE AMES *SALMONELLA TYPHIMURIUM* HISTIDINE
REVERSION ASSAY FOR VOLATILE CHEMICALS, WITH AND
WITHOUT METABOLIC ACTIVATION**

Genesys Research Incorporated's portion of the above titled study was reviewed for compliance with Quality Assurance (QA) regulations and with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The practices used in the study were found to be in compliance with these regulations.

 12/17/94

Ann D. Mitchell, Ph.D. Date
Study Director

**GENESYS RESEARCH INCORPORATED'S
QUALITY ASSURANCE STATEMENT**

With the exception of the handling, storage, dilution (for exposure of the bacteria) and analytical chemistry of the test material, which were the responsibility of ManTech Environmental Technology, Incorporated, the data and the report for the following study carried out at Genesys Research, Incorporated has been reviewed and approved for compliance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The final report accurately describes the methods that were used and accurately reflects the raw data of the study.

ManTech Environmental Technology Incorporated Study Number: 1093-F30

Genesys Research, Incorporated Study Number: 94035

Type Study: *Salmonella typhimurium* histidine reversion assay

Protocol Signed by Study Director: March 19, 1994

Date Testing Started: March 29, 1994

Critical Phase Audit(s): March 29 and May 25, 1994

Date Testing Completed: May 25, 1994

Date Draft Report Audited: August 26, 28 and 29, 1994

Date Audit Findings Reported to Management: March 29, May 25 and September 9, 1994

Approved: Helen M. King
Helen M. King, B.S.
Quality Assurance Officer for Genesys

Date: 12/17/94

**MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

Study Title: *In Vitro* and Inhalation Toxicity Study of Iodotrifluoromethane

Project Number: 1093-F30

Study Director: Allen Ledbetter

ManTech Environmental Technology's portion of this study was conducted in accordance with EPA Good Laboratory Practice Regulations (GLP) as set forth in the Code of Federal Regulations (40 CFR 792). There were no significant deviations, in the work conducted by ManTech, from the aforementioned GLP regulations that would have affected the integrity of the study or the interpretation of the test results. The ManTech generated raw data have been reviewed by the Study Director, who certifies that the information contained in this report represents an appropriate and accurate conclusion within the context of the study design and evaluation criteria. Deviations are listed below:

1. The sponsor was responsible for the test substance characterization, stability and homogeneity analysis.

All original ManTech generated raw data are retained in the ManTech Environmental Technology's Archives, at 5 Triangle Drive, Research Triangle Park, NC 27709, with a copy of the final study report.

SUBMITTED BY:

Study Director:


Allen Ledbetter Date

**MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED
QUALITY ASSURANCE STATEMENT**

Study Title: *In Vitro* and Inhalation Toxicity Study of Iodotrifluoromethane

Project Number: 1093-F30

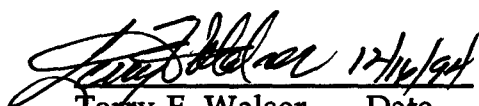
Study Director: Allen Ledbetter

Report Audit Dates:

This study has been subjected to inspections and the report has been audited by ManTech Environmental Technology's Quality Assurance Unit. The report describes the methods and procedures used in the study and the reported results accurately reflect ManTech's raw data. ManTech's raw data and a copy of the final report will be stored in room 210 in the MET building at Research Triangle Park, NC. The sponsor was responsible for the Iodotrifluoromethane characterization, stability and homogeneity analyses.

The following are the inspection dates, and the dates inspection reports were submitted:

<u>Phase(s)</u>	<u>Date(s) of Inspection</u>	<u>Report Submitted to Study Director</u>	<u>Report Submitted to Management</u>
Protocol	7/29/94	7/29/94	7/29/94
Final Data Audit	12/15/94	12/16/94	12/16/94


Terry F. Walser Date
Quality Assurance Officer

**MUTAGENESIS TESTING OF IODOTRIFLUOROMETHANE (CF₃I)
USING THE AMES *SALMONELLA TYPHIMURIUM* HISTIDINE
REVERSION ASSAY FOR VOLATILE CHEMICALS, WITH AND
WITHOUT METABOLIC ACTIVATION**

1. INTRODUCTION

Under subcontract to ManTech Environmental Technology, Incorporated (ManTech), Dayton, Ohio (ManTech/Dayton) Genesys Research, Incorporated (Genesys) used the exposure chamber modification of the *Salmonella typhimurium* histidine (*his*) reversion mutagenesis assay to examine the potential of iodotrifluoromethane (CF₃I) to induce frame shift and base pair substitution *his*⁻ → *his*⁺ reversion mutations. Allen Ledbetter, ManTech Environmental Technology, Incorporated, Research Triangle Park, North Carolina (ManTech/RTP), was responsible for handling, storage, dilution (for exposure of the bacteria), and analytical chemistry of the test material.

Testing at Genesys consisted of all procedures not performed by ManTech/RTP and was conducted under the direction of Ann D. Mitchell, Ph.D., Study Director, by J. Thom Deahl, M.S., and Diane M. Brecha, B.S., Genetic Toxicologists, in accordance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97). Testing for this study was initiated with a preliminary concentration range-finding assay on March 29, 1994 and concluded on May 25, 1994 with mutagenesis assay plate counts. The protocol, a protocol amendment, raw data obtained by Genesys, and a copy of this report will be retained in Genesys' archives located at 2300 Englert Drive, Durham, NC 27713.

2. BACKGROUND

Evidence suggests that a high percentage of chemicals that elicit a mutagenic response in the *Salmonella* assay are potential animal and human mutagens and carcinogens (McCann et al., 1975a; McCann and Ames, 1976; Sugimura et al., 1976; Tennant et al., 1987). Because the *Salmonella* assay is efficient, can indicate mechanisms of chemical interaction with DNA, and produces few positive results for noncarcinogens, it is the cornerstone of evaluations for genotoxicity.

The *Salmonella typhimurium* strains used for the reverse mutation assay are histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only those cells that revert to histidine independence are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The histidine revertants are easily visible as colonies against the slight background growth. The sponta-

neous reversion rate of each strain is relatively constant (Maron and Ames, 1983; McCann et al., 1975a), but when a mutagen is added to the agar, the mutation rate is increased, usually in a dose-related manner.

Strains TA1535 and TA100 are reverted to histidine independence by many mutagens that cause base-pair substitutions; strains TA1537, TA1538 and TA98 are reverted by many frameshift mutagens. In addition to having mutations in the histidine operon, all the indicator strains have a mutation (*rfa*) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (uv)-induced DNA damage (*uvrB*). The *rfa* mutation makes the strains more permeable to many large molecules. The *uvrB* mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents.

Strains TA100 and TA98 are derived from strains TA1535 and TA1538, respectively, and contain the resistance transfer factor, plasmid pKM101, which is believed to cause an additional increase in error-prone DNA repair, leading to even greater sensitivity to most mutagens (Ames et al., 1975, Maron and Ames, 1983, McCann et al., 1975b). In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker for detecting the presence of plasmid pKM101 in these strains.

In the standard plate incorporation *Salmonella* histidine reversion assay, the test material, bacteria, and either a metabolic activation mixture (S9) or buffer are added to liquid top agar which is mixed then immediately poured on a plate of bottom agar. However, volatile, water-insoluble compounds may be poorly detected in the standard plate incorporation assay and may be tested, instead, with a preincubation modification (Maron and Ames, 1983). For this approach, the test material, bacteria, and S9 (if used) are incubated for 20-30 min. at 37°C, then top agar is added, and the mixture is poured on a plate of bottom agar. Increased activity with preincubation is attributed to the fact that the test compound, bacteria, and S9 are incubated at higher concentrations (without agar present) than in the standard plate incorporation test.

In contrast to the plate incorporation assay and the preincubation modification, test materials which are volatile at ambient temperature are tested by the exposure chamber modification (Zeiger et al., 1992). In this procedure, the *Salmonella* strains and S9 mix, or buffer, are incorporated into the top agar then poured onto the minimal agar plates, the plates are stacked in an exposure chamber, and the air is evacuated. A measured amount of the test gas is introduced and air is allowed to flow in until atmospheric pressure is reached. The chamber is then sealed and placed at 37°C for 48 hr. At the end of this period, the exposure chamber is removed to a 100%-exhausted laminar flow hood, the lid removed, and the residual test gas allowed to dissipate for at least 20-30 minutes. The plates are then removed from the exposure chamber, and the colonies are counted.

Because CF₃I is a volatile material, it was tested by the exposure chamber modification, and plastic Billups-Rothenberg exposure chambers were used for the exposure periods.

3. METHODS

3.1. Identification, Storage, and Dilution of the Test Material

The test material, iodotrifluoromethane (CF₃I; molecular weight 195.91; CAS Number 2314-97-8), a colorless gas, was received in steel gas cylinders from ManTech/Dayton on February 27, 1994 and on March 3, 1994 transferred to Allen Ledbetter, ManTech/RTP, who was responsible for handling, storage, and dilution of the test material. The CF₃I was stored at ManTech/RTP in the original containers at room temperature (approximately 72°F). ManTech/Dayton documented the strength, purity, and composition of the test material and provided a Material Safety and Data Sheet (MSDS) from Pacific Scientific for CF₃I. Upon acceptance of the final report, the remaining test material will be returned to the Sponsor. No reserve sample will be retained by ManTech/RTP.

3.2. Tester Strains

The tester strains used in this study were TA1535, TA1537, TA1538, TA98, and TA100, obtained from Dr. Bruce Ames of the University of California at Berkeley (Ames *et al.*, 1975). These indicator strains are kept frozen (-80°C) in nutrient broth supplemented with 8.3% sterile glycerol in 1.8-ml aliquots containing about 4×10^8 cells/ml that had been checked for their genotypic characteristics (*his*, *rfa*, *uvrB*, *bio*) and for the presence of the plasmid. Before each experiment, the cultures were grown overnight with shaking at 37°C in Oxoid No. 2 broth.

3.3. Metabolic Activation System

Rat liver S9 homogenate, in KCl buffer, prepared aseptically from Aroclor 1254-induced male Sprague-Dawley rats, was obtained from Molecular Toxicology, Inc., Annapolis, MD, and stored frozen in liquid nitrogen. The sterile S9 was thawed and used to prepare an S9 mixture immediately before the chemical exposure step of each assay by addition of the homogenate to a mixture of cofactors sterilized by filtration. Therefore, no filtration of the S9 homogenate or S9 mixture was required. The final concentrations of the S9 mixture were 50 µl/ml S9 homogenate, 4 µmol/ml NADP, 5 µmol/ml D-glucose-6-phosphate, 8 µmol/ml MgCl₂, 33 µmol/ml KCl, and 100 µmol/ml sodium phosphate buffer (pH 7.4).

3.4. Controls

Concurrent sterility, negative, and positive controls were used in each assay. Sterility controls included separately plating out the top agar, metabolic activation mixture, and buffer. For negative controls, plates containing bacteria, top agar and buffer or, for tests with metabolic activation, the metabolic activation mixture were exposed to filtered air.

The following positive control chemicals were used: sodium azide (CAS No. 26628-22-8) for the base-pair substitution mutants TA1535 and TA100; 9-aminoacridine (CAS No.

90-45-9) for the frameshift mutant TA1537; 4-nitro-*o*-phenylenediamine (CAS No. 99-56-9) for the frameshift mutants TA98 and TA1538; and 2-anthramine (CAS No. 613-13-8) for all tester strains in the presence of metabolic activation.

3.5. General Procedure

The specific test method has been described in detail (Ames et al., 1975; Maron and Ames, 1983). The exposure chamber assay for each sample was performed in the following way. To a sterile glass test tube were added: 0.1 ml of indicator organisms (about 10^8 bacteria), 0.5 ml of the metabolic activation mixture or buffer, and 2.00 ml of molten 0.615% agar. In addition, 0.1 ml of the appropriate positive control was added to each positive control plate. This top agar mixture was then vortexed gently and poured onto a prelabeled plate containing about 25 ml of minimal glucose agar plus biotin.

After the top agar had set, the test material (or negative control) plates for all five strains that were to be exposed to one concentration of the test material (or to air, the negative control) were placed on a shelf on the bottom section of plastic exposure chamber (Modular Incubator Chamber, Billups-Rothenberg, Del Mar, CA) with an internal volume of approximately 1 liter. The chambers, which consisted of an upper and lower section connected by a gasket and a large stainless steel adjustable squeeze clamp, were then closed by connecting and sealing the upper and lower sections.

The exposure chambers were transported from Genesys to ManTech/RTP where they were labeled as to the desired concentrations; then gas was introduced into an inlet port in the bottom section of each chamber, circulated throughout the chamber, and drawn out of an exhaust port, which was also located in the bottom section. (See Exposure Method, below.) Chamber atmosphere samples, for infrared (IR) analysis were collected from the exhaust port.

The chambers containing the plates were then returned to Genesys where they were incubated at 37°C for approximately 48 hours. Exposure chambers were not required for plates of bacteria treated with positive controls, which were also incubated at 37°C for approximately 48 hours. Following the exposure period, the plates were removed from the exposure chambers, and the number of histidine independent revertant colonies on each plate was counted using an Artek 982B colony counter, or by hand if the colonies could not be counted accurately with the Artek.

3.6. Exposure Method

The test material and dilution air were metered through calibrated flowmeters into the exposure chambers, and chamber atmosphere samples were collected, via a gas tight syringe, and injected into the IR instrument for analysis. The test material and the dilution air were adjusted until the desired chamber concentration was obtained. The chamber exhaust was first disconnected, and then the gases were shut off. This was done to prevent diluting the chamber atmosphere. The inlet and exhaust ports were then sealed with screw clamps. During the mutagenesis assay, two chambers per exposure level were required to hold all the petri plates. To ensure that both chambers

received the same concentration, the gas was introduced to the inlet port of one chamber which was exhausted into the inlet of the second chamber. The exhaust from the second chamber was then analyzed by IR.

3.7. Analysis of Chamber Atmospheres

Prior to analyzing the chamber atmospheres, the IR instrument (Miran 1A, Foxboro Corp., Foxboro, MA) was calibrated using a "closed-loop" method. IR instrument operating parameters were: wavelength, 9.7 microns; pathlength, 9.75 meters; absorbance, 0.25; slit, 2; and range, X1. A calibration curve was prepared using concentration versus recorder chart lines. A Texas TI-60 calculator (Texas Instruments, Lubbock, TX) was used to determine the calibration curve using a least-squares method. The chamber atmospheres were then analyzed by withdrawing a volume of the atmosphere with a gas-tight syringe and injecting it into the IR instrument which was in the "closed-loop" configuration. The number of chart lines was entered into the calculator and the corresponding concentration obtained. The concentration was then corrected for the injection volume.

3.8. Preliminary Range-Finding Assays

Two preliminary assays of CF₃I were conducted with strain TA100 in the presence and absence of metabolic activation to determine a suitable nontoxic dose range for the mutagenicity assay. The results of each testing condition, without and with activation, were evaluated separately. Toxic concentrations were defined as those that produced a decrease in the number of colonies, or a clearing in the density of the background lawn, or both.

3.9. Mutagenesis Assay

Once a dose range had been established, CF₃I was assayed utilizing the five tester strains (TA1535, TA1537, TA1538, TA98, and TA100) over 5 dilutions of the test material such that the highest concentration would be one expected to result in toxicity or would be the highest concentration that could be tested. The mutagenesis assay was conducted using three plates per dose level, in the presence and absence of S-9 metabolic activation. The procedures used were the same as in the preliminary assay except that, because 30 plates were exposed to each test material concentration (5 strains x 3 plates/concentration x 2 [without and with activation]), two chambers, connected in series, were used to hold the 30 plates per concentration.

3.10. Raw Data Collection

All observations, raw data collected, and calculations were recorded onto standard forms which were bound together with the study protocol at the conclusion of testing.

The actual number of revertant colonies was reported for each plate, and the mean number of revertant colonies per plate and the standard deviation were reported for

each concentration of the test material and for the positive and negative controls. Concentrations that resulted in a mutagenic response were indicated with a ♦.

3.11. Analysis and Interpretation of Results

The data generated were considered acceptable if the controls were within the laboratory's historical ranges and if a sufficient number of nontoxic concentrations were tested to determine if a test material were capable of inducing a dose-related mutagenic response.

A test material is considered mutagenic for a condition and strain if a dose-related increase in the number of revertants is observed over three concentrations and the highest increase in strains TA1535, TA1537 and TA1538 is equal to three times the solvent control value or the highest increase in strains TA98 and TA100 is equal to two times the solvent control value (Brusick in Hayes, 1989). A test material is considered negative if the criteria for a positive response are not met and the positive control values are within the historical range for the laboratory.

Both biological and statistical significance were considered together in the evaluation of the results; the final interpretation of the results was the responsibility of the Study Director.

4. RESULTS AND DISCUSSION

The analysis of the exposure chamber atmospheres indicated that the desired concentrations were achieved within 30%, as summarized in Table 1.

The results obtained for testing CF₃I in the *Salmonella typhimurium* histidine reversion exposure chamber assays are presented in Tables 2 - 4. All positive and negative control values were within the laboratory's appropriate ranges, which are consistent with the ranges reported in the literature, and a sufficient number of non-toxic concentrations were tested in the mutagenesis assay; therefore, the data were acceptable.

CF₃I was tested in two preliminary assays (Tables 2 and 3) and one mutagenesis assay (Table 4). In the initial preliminary assay (Table 2), CF₃I was tested over 8 concentrations ranging from 116,048 to 805,973 ppm CF₃I, and the colony counts in TA100 were increased by less than two times the solvent control value at 116,048 ppm CF₃I in the absence of activation and at concentrations up to 248,458 ppm CF₃I, in the presence of activation. However, toxicity as indicated by a decrease in the number of colonies was obtained at concentrations of CF₃I \geq 181,193 ppm in the absence of activation and at concentrations of CF₃I \geq 347,843 ppm in the presence of activation, and toxicity as indicated by a clearing in the density of the background lawn was noted for all concentrations tested in the absence and presence of activation. Because excessive toxicity was obtained in this range-finding assay of CF₃I, the range-finding assay was repeated.

In the repeat of the preliminary assay (Table 3), CF₃I was tested in TA100 over 5 concentrations ranging from 1,253 to 94,873 ppm, and toxicity as indicated by a slight reduction in the density of the background lawn was observed only at the highest concentration tested, without and with activation. In this assay, a clear concentration-related increase in TA100 revertant colonies was observed over all concentrations tested, with the increase essentially as high as that of the positive controls at 94,873 ppm CF₃I, in both the absence and presence of activation.

Concentrations tested in the mutagenesis assay of CF₃I (Table 4) ranged from 1,060 to 85,908 ppm CF₃I, or approximately the concentration range that was tested in the second preliminary assay. Toxicity as indicated by a slight reduction in the background lawn was noted only for strain TA98 in the absence of metabolic activation, but toxicity as indicated by a decrease in the number of colonies was observed at the highest concentration tested, 85,908 ppm CF₃I, for strain TA1537 in the presence of activation and strains TA1535 and TA100 in the absence and presence of activation.

As summarized in Table 4, CF₃I induced a positive mutagenic response as indicated by a concentration-related increase in mean histidine revertant colonies/plate, in the absence and presence of activation, in four of the five tester strains. Of the three strains that detect frameshift mutations, TA1538 was negative without and with activation; TA1537 yielded a positive response at ~5X background (the negative control) without activation and ~3X background with activation, and TA98 yielded a positive responses that were ~4X and ~3X background without and with activation, respectively. More pronounced positive responses were obtained in the two strains that detect base pair substitution mutations. In strain TA1535, revertant colonies were ~60X background without and with activation, with both responses higher than the respective positive controls. In strain TA100, the number of revertant colonies was ~12 background without activation and ~8.5X background with activation. Thus without and with activation, CF₃I was mutagenic inducing frameshift and, particularly, base-pair mutations in *Salmonella typhimurium*.

5. REFERENCES

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Table 1

**COMPARISON OF NOMINAL AND IR DETERMINED CONCENTRATIONS
OF IODOTRIFLUOROMETHANE (CF₃I) IN THE
SALMONELLA TYPHIMURIUM ASSAYS**

	<u>Desired Concentration(ppm)</u>	<u>IR Determined Concentration (ppm)</u>	<u>% of Desired</u>
Preliminary Assay	100,000	116,048	116.0
	200,000	181,193	90.6
	300,000	248,458	82.8
	400,000	347,843	87.0
	500,000	464,736	92.9
	600,000	529,640	88.3
	700,000	667,806	95.4
	800,000	805,973	100.7
Repeat of Preliminary Assay	1,000	1,253	125.3
	3,200	3,037	94.9
	10,000	8,634	86.3
	32,000	33,657	105.2
	100,000	94,873	94.9
Mutagenesis Assay	1,000	1,060	106.0
	3,200	2,775	86.7
	10,000	10,586	105.9
	32,000	23,230	72.6
	100,000	85,908	85.9

Table 2

**PRELIMINARY SALMONELLA TYPHIMURIUM ASSAY OF
IODOTRIFLUOROMETHANE (CF₃I)**

<u>Chemical</u>	<u>S9</u>	<u>Dose Per Plate</u>	TA100 Histidine Revertant <u>Colonies/Plate</u>			<u>Mean ± S.D.</u>			<u>Notes</u>
Air	-	N/A	268 252	288 292	248 253	267	±	19.2	4+
CF ₃ I	-	116,048 ppm	362	396	304	354	±	46.5	SR
	-	181,193 ppm	270	197	181	216	±	47.4	SR
	-	248,458 ppm	293	225	187	235	±	53.7	SR
	-	347,843 ppm	183	86	104	124	±	51.6	R
	-	464,736 ppm	128	138	147	138	±	9.5	R
	-	529,640 ppm	102	7	10	40	±	54.0	R
	-	667,806 ppm	0	0	0	0	±	0.0	R
	-	805,973 ppm	0	0	0	0	±	0.0	R
Sodium azide	-	1.5 µg	628	634	602	621	±	17.0	◆
Air	+	N/A	231 217	246 184	223 216	220	±	20.6	4+
CF ₃ I	+	116,048 ppm	420	516	431	456	±	52.5	◆,SR
	+	181,193 ppm	412	396	378	395	±	17.0	SR
	+	248,458 ppm	424	337	279	347	±	73.0	R
	+	347,843 ppm	134	193	187	171	±	32.5	R
	+	464,736 ppm	187	225	224	212	±	21.7	R
	+	529,640 ppm	169	164	150	161	±	9.8	R
	+	667,806 ppm	0	0	0	0	±	0.0	R
	+	805,973 ppm	0	0	0	0	±	0.0	R
2-Anthramine	+	2.5 µg	1,055	1,059	955	1,023	±	58.9	◆

4+ = Normal background lawn

R = Reduced background lawn

SR = Slightly reduced background lawn

◆ = Positive

Table 3

**REPEAT OF PRELIMINARY *SALMONELLA TYPHIMURIUM* ASSAY OF
IODOTRIFLUOROMETHANE (CF₃I)**

Chemical	S9	Dose Per Plate	TA100 Histidine Revertant Colonies/Plate			Mean ± S.D.		Notes
Air	-	N/A	84 97	110 84	90 91	93 ±	9.8	
CF ₃ I	-	1,253 ppm	175	185	126	162 ±	31.6	
	-	3,037 ppm	285	276	253	271 ±	16.5	◆
	-	8,634 ppm	685	588	630	634 ±	48.6	◆
	-	33,657 ppm	893	1,079	1,011	994 ±	94.1	4+,◆
	-	94,873 ppm	1,400	1,407	1,393	1,400 ±	7.0	SR,◆
Sodium azide	-	1.5 µg	1,614	1,248	1,389	1,417 ±	184.6	◆
Air	+	N/A	130 137	139 127	131 118	130 ±	7.5	
CF ₃ I	+	1,253 ppm	153	117	144	138 ±	18.7	
	+	3,037 ppm	208	234	215	219 ±	13.5	
	+	8,634 ppm	481	506	491	493 ±	12.6	◆
	+	33,657 ppm	625	854	890	790 ±	143.7	4+,◆
	+	94,873 ppm	1,367	1,342	1,171	1,293 ±	106.7	SR,◆
2-Anthramine	+	2.5 µg	1,422	1,469	2,227	1,706 ±	451.8	◆

4+ = Normal background lawn

SR = Slightly reduced background lawn

◆ = Positive

Table 4

**SALMONELLA TYPHIMURIUM MUTAGENESIS ASSAY OF
IODOTRIFLUOROMETHANE (CF₃I)**

Chemical	S9	Dose Per Plate	Strain	Histidine Revertant Colonies/Plate			Mean	±	S.D.	Notes
Air	-	N/A	TA1535	23 22	25 27	18 23	23	±	3.0	
CF ₃ I	-	1,060 ppm	TA1535	94	76	71	80	±	12.1	◆
	-	2,775 ppm	TA1535	158	186	174	173	±	14.0	◆
	-	10,586 ppm	TA1535	457	444	664	522	±	123.4	◆
	-	23,230 ppm	TA1535	1,386	1,403	1,227	1,395	±	97.1	◆
	-	85,908 ppm	TA1535	335	440	248	341	±	96.1	4+,◆
Sodium azide	-	1.5 µg	TA1535	562	570	598	577	±	18.9	◆
Air	+	N/A	TA1535	16 23	14 17	9 17	16	±	4.6	
CF ₃ I	+	1,060 ppm	TA1535	57	25	40	41	±	16.0	
	+	2,775 ppm	TA1535	84	132	140	119	±	30.3	◆
	+	10,586 ppm	TA1535	257	279	264	267	±	11.2	◆
	+	23,230 ppm	TA1535	1,259	810	677	915	±	305.0	◆
	+	85,908 ppm	TA1535	452	442	608	501	±	93.1	4+,◆
2-Anthramine	+	2.5 µg	TA1535	310	204	314	276	±	62.4	◆
Air	-	N/A	TA1537	8 12	16 8	8 6	10	±	3.7	
CF ₃ I	-	1,060 ppm	TA1537	5	13	10	9	±	4.0	
	-	2,775 ppm	TA1537	9	14	18	14	±	4.5	
	-	10,586 ppm	TA1537	17	22	23	21	±	3.2	
	-	23,230 ppm	TA1537	30	39	28	32	±	5.9	◆
	-	85,908 ppm	TA1537	49	61	36	49	±	12.5	4+,◆
9-AA	-	100.0 µg	TA1537	1,089	471	487	682	±	352.3	◆
Air	+	N/A	TA1537	5 8	10 5	14 13	9	±	3.9	
CF ₃ I	+	1,060 ppm	TA1537	9	13	16	13	±	3.5	
	+	2,775 ppm	TA1537	12	14	5	10	±	4.7	
	+	10,586 ppm	TA1537	12	21	14	16	±	4.7	
	+	23,230 ppm	TA1537	27	26	36	30	±	5.5	◆
	+	85,908 ppm	TA1537	19	26	22	22	±	3.5	4+
2-Anthramine	+	2.5 µg	TA1537	150	137	199	162	±	32.7	◆

Table 4 (continued)

Chemical	S9	Dose Per Plate	Histidine Revertant			Mean	±	S.D.	Notes	
			Strain	Colonies/Plate						
Air	-	N/A	TA1538	22 16	18 21	26 28	22	±	4.6	
CF ₃ I	-	1,060 ppm	TA1538	18	23	21	21	±	2.5	
	-	2,775 ppm	TA1538	23	36	30	30	±	6.5	
	-	10,586 ppm	TA1538	12	22	16	17	±	5.0	
	-	23,230 ppm	TA1538	36	36	34	35	±	1.2	
	-	85,908 ppm	TA1538	14	21	25	20	±	5.6	4+
4-NOPD	-	2.0 µg	TA1538	235	244	245	241	±	5.5	◆
Air	+	N/A	TA1538	26 18	31 19	30 30	26	±	5.8	
CF ₃ I	+	1,060 ppm	TA1538	12	18	19	16	±	3.8	
	+	2,775 ppm	TA1538	10	18	16	15	±	4.2	
	+	10,586 ppm	TA1538	34	26	28	29	±	4.2	
	+	23,230 ppm	TA1538	23	21	22	22	±	1.0	
	+	85,908 ppm	TA1538	37	30	32	33	±	3.6	4+
2-Anthramine	+	2.5 µg	TA1538	838	1,097	856	930	±	144.6	◆
Air	-	N/A	TA98	25 25	26 14	16 23	22	±	5.2	
CF ₃ I	-	1,060 ppm	TA98	30	19	36	28	±	8.6	SR
	-	2,775 ppm	TA98	26	35	31	31	±	4.5	SR
	-	10,586 ppm	TA98	57	56	45	53	±	6.7	SR,◆
	-	23,230 ppm	TA98	68	99	97	88	±	17.3	SR,◆
	-	85,908 ppm	TA98	107	52	106	88	±	31.5	4+,◆
4-NOPD	-	2.0 µg	TA98	233	191	208	211	±	21.1	◆
Air	+	N/A	TA98	32 34	30 34	28 30	31	±	2.4	
CF ₃ I	+	1,060 ppm	TA98	31	56	26	38	±	16.1	
	+	2,775 ppm	TA98	39	39	36	38	±	1.7	
	+	10,586 ppm	TA98	56	58	59	58	±	1.5	
	+	23,230 ppm	TA98	107	93	76	92	±	15.5	◆
	+	85,908 ppm	TA98	145	84	72	100	±	39.1	4+,◆
2-Anthramine	+	2.5 µg	TA98	1,148	1,041	915	1,035	±	116.6	◆

Table 4 (concluded)

Chemical	S9	Dose Per Plate	Histidine Revertant			Mean	±	S.D.	Notes	
			Strain	Colonies/Plate						
Air	-	N/A	TA100	89	90	106	96	±	10.1	
				89	92	112				
CF ₃ I	-	1,060 ppm	TA100	143	112	136	130	±	16.3	
	-	2,775 ppm	TA100	165	293	244	234	±	64.6	◆
	-	10,586 ppm	TA100	477	491	558	509	±	43.3	◆
	-	23,230 ppm	TA100	1,063	1,235	1,107	1,135	±	89.4	◆
	-	85,908 ppm	TA100	438	619	589	549	±	97.0	4+,◆
Sodium azide	-	1.5 µg	TA100	1,306	968	1,040	1,105	±	178.0	◆
Air	+	N/A	TA100	105	119	105	111	±	6.5	
				116	106	116				
CF ₃ I	+	1,060 ppm	TA100	134	134	123	130	±	6.4	
	+	2,775 ppm	TA100	249	236	226	237	±	11.5	◆
	+	10,586 ppm	TA100	492	428	543	488	±	57.6	◆
	+	23,230 ppm	TA100	834	927	1,046	936	±	106.3	◆
	+	85,908 ppm	TA100	747	815	828	797	±	43.5	4+,◆
2-Anthramine	+	2.5 µg	TA100	1,482	1,343	1,318	1,381	±	88.4	◆

4NOPD = 4-Nitro-*o*-phenylenediamine

9-AA = 9-Aminoacridine

♦ = Positive

4+ = Normal background lawn

R = Reduction in background lawn

SR = Slight reduction in background lawn